7. Norris, D.R., Marra, P.P., Kyser, T.K., Sherry, T.W., and Ratcliffe, L.M. (2004). Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. Proc. R. Soc. B *271*, 59–64.
8. Reudink, M.W., Marra, P.P., Kyser, T.K., Boag, P.T., Langin, K.M., and Ratcliffe, L.M. (2009). Non-breeding season events influence sexual selection in a long-distance migratory bird. Proc. R. Soc. B *276*, 1619–1626.
9. Møller, A.P., and Szép, T. (2005). Rapid evolutionary change in a secondary sexual character linked to climatic change. J. Evol. Biol. *18*, 481–495.
10. Scordato, E.S.C., Bontrager, A.L., and Price, T.D. (2012). Cross-generational effects of climate change on expression of a sexually selected trait. Curr. Biol. *22*, 78–82.
11. Marchetti, K. (1998). The evolution of multiple male traits in the yellow-browed leaf warbler. Anim. Behav. *55*, 361–376.
12. Both, C., Artemyev, A.V., Blaauw, B., Cowie, R.J., Dekhuijzen, A.J., Eeva, T., Enemar, A., Gustafsson, L., Ivankina, E.V., Järvinen, A., *et al*. (2004). Large-scale geographic variation confirms that climate change causes birds to lay earlier. Proc. R. Soc. Lond. B *271*, 1657–1662.
13. Verhulst, S., and Nilsson, J.-A. (2008). The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. Phil. Trans. R. Soc. B *363*, 399–410.
14. Visser, M.E., Both, C., and Lambrechts, M.M. (2004). Global climate change leads to mistimed avian reproduction. Adv. Ecol. Res. *35*, 89–110.
15. Thomas, D.W., Blondel, J., Perret, P., Lambrechts, M.M., and Speakman, J.R. (2001). Energetic and fitness costs of mismatching resource supply and demand in seasonally breeding birds. Science *291*, 2598–2600.
16. Chaine, A.S., and Lyon, B.E. (2008). Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. Science *319*, 459–462.
17. Cockburn, A., Osmond, H.L., and Double, M.C. (2008). Swingin' in the rain: condition dependence and sexual selection in a capricious world. Proc. R. Soc. B *275*, 605–612.
18. Slagsvold, T., Lifjeld, J.T., Stenmark, G., and Breiehagen, T. (1988). On the cost of searching for a mate in female pied flycatchers *Ficedula hypoleuca*. Anim. Behav. *36*, 433–442.
19. Vitousek, M.N. (2009). Investment in mate choice depends on resource availability in female Galapagos marine iguanas *(Amblyrhynchus cristatus)*. Behav. Ecol. Sociobiol. *64*, 105–115.
20. Nussey, D.H., Postma, E., Gienapp, P., and Visser, M.E. (2005). Selection on heritable phenotypic plasticity in a wild bird population. Science *310*, 304–306.

Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, 80309, USA.
*E-mail: maren.vitousek@colorado.edu

DOI: 10.1016/j.cub.2011.12.024

Developmental Biology: Taking Flight

Powered flight was first mastered by insects, many millions of years ago. Now, studies with the fruit fly *Drosophila melanogaster* reveal the critical role of a conserved transcription factor in programming the development of specialized flight muscles.

Sudipto Roy[1] and K. VijayRaghavan[2]

"God in his wisdom made the fly, And then forgot to tell us why." It must have been an annoying buzzing that brought Ogden Nash to pen his famous poem. But the flight manoeuvres of insects, and in particular flies, are also sophisticated: they hover; rapidly change direction, dive and even fly backwards. Anatomically, insect flight probably first evolved by muscles inserting into the wing hinge: mayflies and dragonflies are extant examples where flight is powered by such 'direct' flight muscles. In most insects, however, flight is powered by controlling wing oscillation differently, namely through indirect flight muscles (Figure 1A). They are called 'indirect' because the muscles insert into the thoracic exoskeleton and produce high frequency wing vibrations by inducing cyclic deformations of the thoracic cuticle and of the wings as an indirect consequence.

Indirect flight muscles also have an unusual physiology: the contraction of one set of muscles stretches another, which in turn causes contraction and stretching of the first set. This results in an oscillation of the thoracic box. The motor neuron's role is to stimulate the muscle periodically, causing the release of Ca^{2+} ions in the muscle, necessary to sustain contraction. The motor neuron firing frequency is asynchronous with indirect flight muscle contraction: the latter can be at several hundred to a 1000 Hz, while the former is usually tens of Hz (Figure 1C). Indirect flight muscles are thus stretch-activated and asynchronous, as distinct from other muscles such as those of the insect leg, which are activated by synchronous neuronal firing.

The unusual physiology of the indirect flight muscles is made possible by their specialized structure in which the muscles are arranged in unaligned fibre bundles, hence the term 'fibrillar muscle', with the endoplasmic reticulum (ER) in the periphery. In contrast, other muscles, such as those of the insect leg, have a more distributed ER and myofibres aligned in a 'tubular' form [1]. While the physiology, ultrastructure and development of indirect flight muscles have been extensively investigated [2,3], the mechanism by which the fibrillar fate is instituted had remained unclear. In a recent paper, Schnorrer and co-authors [4] report that, in *Drosophila*, Spalt major (Salm), a zinc finger transcription factor, functions as a 'master regulator' driving muscle progenitors to differentiate into indirect flight muscles.

An earlier indication for a role of the *salm* gene in indirect flight muscle formation came from a study that screened for genes regulating muscle development in *Drosophila* [5]. The new work [4] now suggests that Salm is a molecular switch that programs the distinctive properties of the indirect flight muscles. Flies deprived of *salm* function in muscle precursors form fewer and abnormal indirect flight muscles whose myofibrillar organization is shifted from fibrillar to tubular. The effect of *salm* was specific for the indirect flight muscles, and the formation and function of the tubular muscles, such as those in the leg, remained unaffected.

In *Drosophila*, embryonic muscle precursors first assemble a set of body wall muscles that allow the larvae to crawl around. Then, during metamorphosis, larval muscles degenerate, and adult muscle precursors fuse and differentiate into new sets of muscles engineered for walking and flight [3]. Indirect flight muscles develop through a precisely choreographed series of events [2,3,6]. The development of one set of indirect flight muscles, the dorsal longitudinal muscles, is rather peculiar in that the adult muscle precursors fuse with three larval muscles that escape

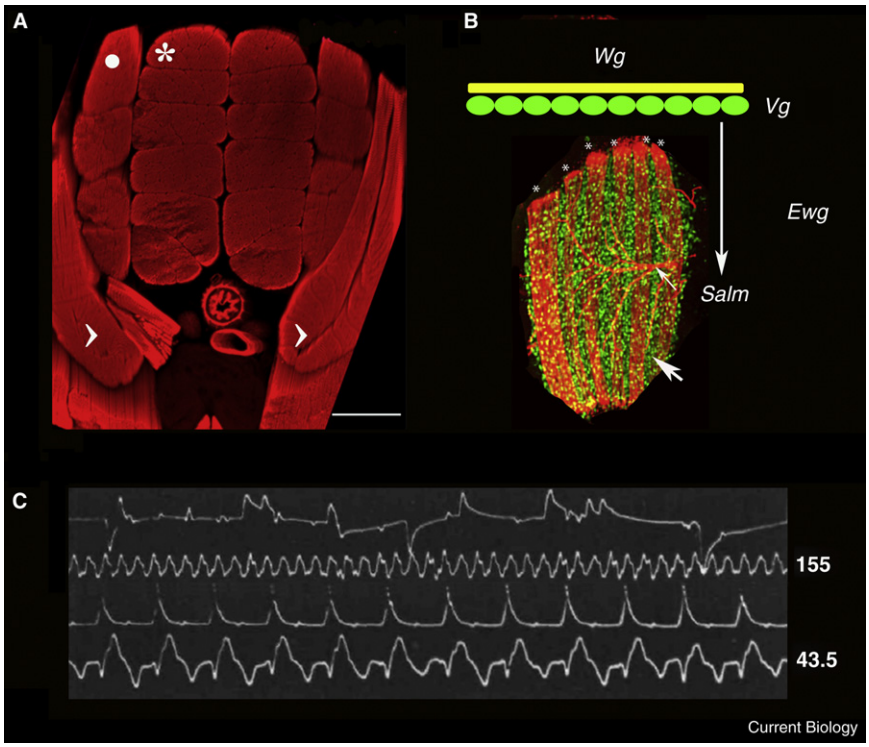

Figure 1. Making flight muscles special with Salm.

(A) Frontal view of the muscles of the *Drosophila* thorax. The dorsal-most fibre bundle of one type of indirect flight muscle is shown, the DVMs (dot). The other DVM fibres are seen below. The other type of indirect flight muscle is the dorsal-longitudinal muscle (asterisk). Below the dorsal longitudinal muscle are the other 5 dorsal longitudinal muscle fibres. The indirect flight muscles (the DVMs and the dorsal longitudinal muscles) are fibrillar, and the other muscles of the thorax, such as the giant jump muscles (marked with >), are tubular. Scale bar = 100 μm. (B) The genetic network underlying indirect flight muscle specification. Wingless (Wg) signaling from the developing thoracic epidermis (yellow bar) signals to underlying adult muscle precursors (green ellipses) which contribute to the indirect flight muscle expressing Vestigial (Vg). Vg in turn acts as a permissive factor for Salm expression in the developing indirect flight muscles. The developing dorsal longitudinal muscles shown in this panel were labeled with mAb22C10 (red), which marks the differentiating fibres (asterisks) and the motor innervation (small arrow). The fusing adult muscle precursors around the differentiating fibres were labeled with antibodies to Erect wing (Ewg, green), a transcription factor that is required for indirect flight muscle development. The relationship of Vg or Salm to Ewg expression is presently not known. (C) The indirect flight muscles oscillate the thoracic exoskeleton rapidly (155 Hz, lower part of the panel), and this is asynchronous with motor neuron firing (upper part of panel). In contrast, the synchronous flight muscles of Lepidoptera, which flaps its wings directly and at a much lower frequency (43.5 Hz, lower part of panel), are in synchrony with neuronal stimulation (upper part of the panel). (Panel A, from M. Umashankar, and illustration of dorsal longitudinal muscles in panel B from Priyankana Mukherjee, NCBS. Panel C is adapted with permission from [1].)

degeneration in each hemithorax, splitting them into six dorsal longitudinal muscle fibers [2,7] (Figure 1A). The first anomaly in indirect flight muscle development in the absence of *salm* is apparent in the failure of these larval muscles to split. In keeping with such indirect flight muscle-specific phenotypes, Schnorrer and colleagues [4] found that the expression of Salm protein is restricted to developing and mature indirect flight muscle fibers. The dynamics of Salm expression in the other set of indirect flight muscles, the dorso-ventral muscles (Figure 1A), which form by the fusion of adult muscle precursors to founder myoblasts [3], is even less clear.

To make sense of how the spatially restricted expression of *salm* expression is controlled, the authors [4] explored the relationship of *salm* expression with that of two other transcription factors, Vestigial (Vg) and Ladybird early (Lbe), which are expressed in the indirect flight muscle and leg muscle precursors, respectively [8,9]. They found that *vg* affected indirect flight muscle development in a manner identical to *salm*, and that *Vg* is required for *salm* expression. In fact, indirect flight muscle defects in *vg* mutant flies could be completely rescued by transgenic provision of *salm* function. However, mis-expression of *vg* itself was not sufficient to switch on *salm* in tubular muscles, and consequently, to transform them to the fibrillar fate. Thus, Vg appears to be a permissive factor for *salm* expression (Figure 1B), a finding that is not too surprising given that Vg expression is observed in the adult muscle precursors well before *salm* expression becomes detectable [8]. How *lbe* influences *salm* expression is less clear. While mis-expression of *lbe* in indirect flight muscle myoblasts repressed *salm*, as the authors concede, this cannot be a universal strategy to keep *salm* switched off in tubular muscles as *lbe* is not expressed in many tubular muscles [9].

Inspired by the function of *salm* in indirect flight muscles, the authors examined whether *salm* is sufficient to program the fibrillar muscle developmental program in tubular muscles. Indeed, mis-expression of *salm* in leg muscles led to a striking conversion to the fibrillar type. A comparison of gene expression profiles of wild-type indirect flight muscles and leg muscles versus *salm*-deficient indirect flight muscles showed a global reduction of indirect flight muscle-specific transcripts, and a concomitant gain of tubular muscle-specific transcripts, in response to the loss of *salm*. What these alterations in gene expression levels and patterns mean for the molecular activity of Salm is up for guesses at the moment. Does Salm, a transcription factor, directly activate indirect flight muscle-specific genes and repress tubular muscle-specific ones or does the regulation involve one or more subordinate transcription factors? With respect to this, it is worth noting that biochemical characterization of the related *Drosophila* Spalt proteins have suggested that they act as transcriptional repressors [10]. Undoubtedly, a lot remains to be learnt about the molecular details of Salm activity in muscle fate determination.

As indirect flight muscles represent a key evolutionary innovation,

Schnorrer and colleagues [4] investigated the function of *salm* in other insects They find that Salm is expressed in the indirect flight muscles of another fly (*Calliphora*), and that RNAi mediated inhibition of Salm activity in a beetle (*Tribolium*) transformed its fibrillar muscles into tubular muscles. Although these data are convincing, analysis of the expression pattern of Salm in *Tribolium* indirect flight muscles would have added further credence to their view. An evolutionary complexity arises from the view [1] that indirect flight muscles evolved several times independently. Are all such indirect flight muscles regulated by Salm? Does Salm regulate flight muscles also in phylogenetically older insects such as mayflies with direct flight muscles?

Finally, we confront yet another evolutionary comparison that is a little more tenuous. The authors wonder whether cardiac abnormalities that have been reported in patients with Townes-Brockes syndrome (TBS), which arise due to mutation of the vertebrate *salm* homologue gene *SALL1* [11], could reflect a conserved role of SALL1 in the specification of vertebrate heart muscle, which, like insect indirect flight muscles, is made up of stretch-activated fibers. Before getting too excited about this possibility, there are a few points worth considering: in mice, where Sall1 expression has been analyzed in some detail, the gene is thought to be transcribed in the endocardial and not the myocardial tissue of the heart [12] . Moreover, mice lacking Sall1 function do not display many of the more prominent clinical features of TBS, such as limb, anorectal or otic abnormalities, and no heart defects have been described [12], although differences in the molecular nature of the mutations in the mouse versus the human gene, as well as redundancy among the many Spalt family members in vertebrates, could account for this discrepancy. Furthermore, despite the apparent phenotypic similarities between many homologous organ systems of flies mutant for *salm* and its sister gene *spalt-related* and individuals afflicted with TBS, there are no reports of any anomaly in the formation or function of the fly heart that relates to the cardiac defects in TBS [13]. Keeping these issues in mind, the idea that *Sall1* has a role in specifying the stretch-activated vertebrate cardiac muscle deserves more detailed scrutiny.

The role for Salm in flight muscles opens exciting new avenues for studying the ways in which insect flight evolved and how flight muscles are specified. Given the inimitability of this discovery, a role for vertebrate Sall genes in cardiac muscle and disease would be the icing on the cake, but isn't the cake a treat even without the icing?

References

1. Pringle, J.W.S. (1981). The evolution of fibrillar muscle in insects. J. Exp. Biol. *94*, 1–14.
2. Fernandes, J., Bate, M., and VijayRaghavan, K. (1991). Development of the indirect flight muscles of Drosophila. Development *113*, 67–77.
3. Roy, S., and VijayRaghavan, K. (1999). Muscle pattern diversification in Drosophila: the story of imaginal myogenesis. Bioessays *21*, 486–498.
4. Schonbauer, C., Distler, J., Jahrling, N., Radolf, M., Dodt, H.U., Frasch, M., and Schnorrer, F. (2011). Spalt mediates an evolutionarily conserved switch to fibrillar muscle fate in insects. Nature *479*, 406–409.
5. Schnorrer, F., Schonbauer, C., Langer, C.C., Dietzl, G., Novatchkova, M., Schernhuber, K., Fellner, M., Azaryan, A., Radolf, M., Stark, A., *et al*. (2010). Systematic genetic analysis of muscle morphogenesis and function in Drosophila. Nature *464*, 287–291.
6. Dong, P.D., Todi, S.V., Eberl, D.F., and Boekhoff-Falk, G. (2003). Drosophila spalt/spalt-related mutants exhibit Townes-Brocks' syndrome phenotypes. Proc. Natl. Acad. Sci. USA *100*, 10293–10298.
7. Dutta, D., Bloor, J.W., Ruiz-Gomez, M., VijayRaghavan, K., and Kiehart, D.P. (2002). Real-time imaging of morphogenetic movements in Drosophila using Gal4-UAS-driven expression of GFP fused to the actin-binding domain of moesin. Genesis *34*, 146–151.
8. Roy, S., and VijayRaghavan, K. (1998). Patterning muscles using organizers: larval muscle templates and adult myoblasts actively interact to pattern the dorsal longitudinal flight muscles of Drosophila. J. Cell Biol. *141*, 1135–1145.
9. Sudarsan, V., Anant, S., Guptan, P., VijayRaghavan, K., and Skaer, H. (2001). Myoblast diversification and ectodermal signaling in Drosophila. Dev. Cell *1*, 829–839.
10. Maqbool, T., Soler, C., Jagla, T., Daczewska, M., Lodha, N., Palliyil, S., VijayRaghavan, K., and Jagla, K. (2006). Shaping leg muscles in Drosophila: role of ladybird, a conserved regulator of appendicular myogenesis. PLoS One *1*, e122.
11. Sanchez, J., Talamillo, A., Gonzalez, M., Sanchez-Pulido, L., Jimenez, S., Pirone, L., Sutherland, J.D., and Barrio, R. (2011). Drosophila Sal and Salr are transcriptional repressors. Biochem. J. *438*, 437–445.
12. Surka, W.S., Kohlhase, J., Neunert, C.E., Schneider, D.S., and Proud, V.K. (2001). Unique family with Townes-Brocks syndrome, SALL1 mutation, and cardiac defects. Am. J. Med. Genet. *102*, 250–257.
13. Nishinakamura, R., Matsumoto, Y., Nakao, K., Nakamura, K., Sato, A., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Scully, S., Lacey, D.L., *et al*. (2001). Murine homolog of SALL1 is essential for ureteric bud invasion in kidney development. Development *128*, 3105–3115.

[1]Institute of Molecular and Cell Biology, Proteos, 61 Biopolis Drive, Singapore 138673, Singapore. [2]National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bellary Road, Bangalore 560065, India.
E-mail: sudipto@imcb.a-star.edu.sg, vijay@ncbs.res.in

DOI: 10.1016/j.cub.2011.12.031